JOURNAL OF BACTERIOLOGY, Jan. 1994, p. 426–431
0021-9193/94/$04.00+0

Vol. 176, No. 2

Glycine Betaine Confers Enhanced Osmotolerance and Cryotolerance on *Listeria monocytogenes*

RINKEI KO,[1] LINDA TOMBRAS SMITH,[2] AND GARY M. SMITH[1]*

Departments of Food Science and Technology[1] and Agronomy and Range Science,[2] University of California, Davis, California 95616

Received 30 August 1993/Accepted 9 November 1993

***Listeria monocytogenes* is a gram-positive food-borne pathogen that is notably resistant to osmotic stress and can grow at refrigerator temperatures. These two characteristics make it an insidious threat to public health. Like several other organisms, *L. monocytogenes* accumulates glycine betaine, a ubiquitous and effective osmolyte, intracellularly when grown under osmotic stress. However, it also accumulates glycine betaine when grown under chill stress at refrigerator temperatures. Exogenously added glycine betaine enhances the growth rate of stressed but not unstressed cells, i.e., it confers both osmotolerance and cryotolerance. Both salt-stimulated and cold-stimulated accumulation of glycine betaine occur by transport from the medium rather than by biosynthesis. Direct measurement of glycine betaine uptake shows that cells transport betaine 200-fold faster at high salt concentration (4% NaCl) than without added salt and 15-fold faster at 7 than at 30°C. The kinetics of glycine betaine transport suggest that the two transport systems are indistinguishable in terms of affinity for betaine and may be the same. Hyperosmotic shock and cold shock experiments suggest the transport system(s) to be constitutive; activation was not blocked by chloramphenicol. A cold-activated transport system is a novel observation and has intriguing implications concerning the physical state of the cell membrane at low temperature.**

Listeria monocytogenes is an opportunistic pathogen that infects primarily pregnant women, the very old or very young, and immunologically compromised individuals, such as people with AIDS or those undergoing corticosteroid therapy (4, 11, 20, 21, 36). The fatality rate among infected individuals is about 25% (34). *L. monocytogenes* is a ubiquitous organism that can be isolated from many sources (22, 41, 42) including food (27), which is the major route of infection in humans. Known outbreaks of listeriosis (16) have been linked to cheese, milk, and coleslaw (16, 33). The largest recent outbreak, which resulted in the deaths of 40 of the 103 to 250 infected people (31), stemmed from contaminated cheese in Los Angeles in 1985 (14). In addition to large outbreaks of listeriosis, sporadic listeriosis occurs nationwide (35) and appears to be a growing public health menace.

Major factors in the recent ascent to prominence of *L. monocytogenes* as a food-borne pathogen are undoubtedly its abilities to grow vigorously at refrigerator temperatures and to grow in osmotically stressful environments (5, 6, 37, 43) such as salted foods and dry surfaces. In fact, there is evidence that it may survive even on particles as dry as dust or flakes of organic material (20). On the other hand, *L. monocytogenes* does not compete well in mixed cultures and is susceptible to bacteriolysins. Food handling and storage practices, such as refrigeration, that eliminate or suppress competitors therefore promote the growth of listeria.

Since high osmolarity and low temperature are conditions that favor *L. monocytogenes* over its competitors, the processes of osmotic adaptation and low temperature adaptation are crucial to its importance as a food-borne pathogen. One mechanism of osmotic stress adaptation commonly found in bacteria involves intracellular accumulation of organic compounds called osmolytes (3, 7, 8, 18, 46), which contribute to a counterbalancing osmotic pressure. Osmolytes are often termed compatible solutes because they do not produce adverse effects upon protein structure and solubility, protein-protein interactions, enzyme-substrate interactions, or protein-nucleic acid interactions, as would high concentrations of inorganic salts (18, 46). Hence, intracellular accumulation of osmolytes, which can occur either by de novo synthesis or by transport from the growth medium, confers tolerance to hyperosmotic stress.

Bacterial adaptation to low temperature, herein termed chill stress, is poorly understood. It is thought to involve modification of membrane lipid composition for the purpose of maintaining optimum membrane fluidity in a process called homeoviscous adaptation (12, 30). However, adaptation to low temperature involves changes in cellular components other than lipids. Cold-inducible proteins of unknown function are known to be expressed in *Escherichia coli* (13) and almost certainly in other organisms as well. In addition, cold intolerance is caused in some organisms by the presence of cold-sensitive enzymes (for an example, see reference 9). In bacteria in which enzyme stability sets the lower limit of growth temperature, cryotolerance could be conferred by the intracellular accumulation of protein-stabilizing molecules, such as osmolytes. This possibility suggests a relationship between psychrotrophy and osmotic tolerance. Here, we report the results of our investigation of this relationship in the osmotically tolerant psychrotroph *L. monocytogenes*.

MATERIALS AND METHODS

L. monocytogenes Scott A was maintained on Trypticase-soy agar slants. Brain heart infusion (BHI; Difco) broth was used as rich medium. When defined medium was required, the medium described by Pine and coworkers (25, 26) minus choline and plus 0.5% glucose (modified Pine's medium) was used. Cell density was determined by turbidity with a Klett-Summerson colorimeter with a green (no. 54) filter.

* Corresponding author. Phone: (916) 752-6168. Fax: (916) 752-4759. Electronic mail: gmsmith@ucdavis.edu.

Salt and cold tolerance on solid medium were determined by using agar plates that contained modified Pine's medium plus 1.5% agar and 4% NaCl with or without 130 μM glycine betaine. Inocula were grown in BHI broth; the cultures were centrifuged at 5,000 × *g* for 10 min, resuspended in modified Pine's medium, and diluted 1:10^6 in modified Pine's medium, and 0.1 ml of the cell suspension was spread on the plates.

Determination of osmolytes in *L. monocytogenes*. Inocula were grown in BHI broth and inoculated (1%) into BHI broth with or without 8% NaCl. The cultures were grown at the desired temperature (4 or 30°C) until late log phase (95 to 185 Klett units) and then harvested by centrifugation at 5,800 × *g* for 15 min. The pelleted cells were extracted with perchloric acid, and the resulting extracts were submitted to natural abundance ^{13}C nuclear magnetic resonance (NMR) spectroscopy as previously described (38, 39). Concentrations of osmolytes were determined by comparison of resonance intensities to that of 50 mM alanine, which was added as a standard and the concentration of which was verified by amino acid analysis. Amounts of solutes were normalized to the total cellular protein, which was determined by the method of Lowry et al. (19). Intracellular concentrations were calculated by using the water-accessible cytoplasmic volume as reported by Patchett et al. (23).

Measurement of glycine betaine uptake rates in salt- and cold-adapted cells. *L. monocytogenes* grown in BHI broth was centrifuged at 5,000 × *g* for 10 min, resuspended in modified Pine's medium, and grown at 30°C. This culture was used to inoculate (1%) fresh modified Pine's medium with and without NaCl, as needed, and incubated at the desired temperature. Aliquots of the culture at late log phase were transferred to culture tubes, and 120 μM [^{14}C]glycine betaine (0.02 to 0.05 μCi) was added to determine uptake. At four time points during the linear portion of the reaction, 0.5 ml of the assay mixture was filtered through a 0.44-μm-pore-size membrane (Millipore). The filter was rinsed with medium identical to the growth medium but lacking radioactivity and submitted to liquid scintillation counting in Scintiverse I fluid (Fisher). Uptake rates were normalized to total cellular protein (19). To screen for inhibition of glycine betaine uptake by various betaine analogs and related compounds, transport was measured in the presence of the putative inhibitors at 5 mM, with a glycine betaine concentration of 120 μM.

Observation of transport in osmotically upshocked and cold-shocked cells. *L. monocytogenes* grown in BHI liquid medium was transferred (1%) to modified Pine's medium and grown at 30°C. This culture was diluted into modified Pine's medium containing 120 μM [^{14}C]glycine betaine, and portions were withdrawn, filtered, and counted as described above at various times to determine the rate of uptake. For osmotic upshock experiments, the culture was divided in two, medium containing concentrated NaCl was added to one sample to bring the concentration to 4% NaCl, and the uptake rate was determined. For cold-shock experiments, a portion of the culture was quickly cooled to 7°C and uptake was measured. Before the temperature was decreased (0 to 60 min) the culture grew with a doubling time of 2 h, which accounts for the apparent increase in uptake during that time. Both osmotic-shock and cold-shock experiments were also performed in the presence of 20 and 100 μg of chloramphenicol per ml after a 2-h incubation with the antibiotic.

Determination of kinetic parameters of glycine betaine uptake. Each uptake rate was measured as described above in modified Pine's medium at glycine betaine concentrations between 2 and 30 μM at 2-min intervals for 6 min. For experiments at 30°C, cultures were grown to ~80 Klett units and then diluted in fresh growth medium to 15 to 20 Klett units in order obtain uptake rates in a measurable range. For experiments at 7°C, cultures were grown to 90 Klett units and used without dilution. Kinetic parameters were determined from weighted Lineweaver-Burk plots.

TABLE 1. Osmolytes of stressed *L. monocytogenes* grown in rich medium

Osmolyte	Intracellular concn of osmolyte (mM) at indicated temp and salt level[a]			
	30°C, no added NaCl	30°C, +8% NaCl	4°C, no added NaCl	4°C, +8% NaCl
Glycine betaine	65	1,300	310	1,800
Glutamate	200	640	350	430
Carnitine	120	<100	430	<75

[a] Errors due to noise and loss of sample during workup are 20% or less.

RESULTS

Osmotic adaptation by *L. monocytogenes*. The mechanism of osmotic adaptation in *L. monocytogenes* was investigated by first determining which, if any, osmolytes are accumulated intracellularly in response to salt stress. Perchloric acid extracts of *L. monocytogenes* cultures grown at 30°C in rich medium (BHI) containing 8% NaCl or no added salt were examined by using natural abundance ^{13}C NMR spectroscopy (39). Glycine betaine, one of the more common and most effective osmolytes found in nature (46), was the dominant osmolyte in salt-stressed cells, its concentration increasing by 20-fold over that in unstressed cells (Table 1). ^{13}C NMR spectra of the growth medium indicated the presence of glycine betaine (data not shown), suggesting that intracellular accumulation occurred by transport. The only other major osmolyte observed in extracts of salt-stressed cells was the common metabolite glutamate, which increased by 3.2-fold over that found in extracts of unstressed cells. Carnitine, which was observed in extracts of unstressed cells, was reduced slightly in salt-stressed cells.

To determine whether glycine betaine confers osmotic tolerance to cells growing on a solid (i.e., food-like) surface, 1.5% agar plates containing modified Pine's medium plus 4% NaCl with or without 130 μM glycine betaine were inoculated. After incubation for 36 h at 30°C, about 200 colonies of 1 to 2 mm in diameter were present on plates containing glycine betaine (data not shown), whereas no colonies were visible on plates that did not contain glycine betaine. The presence of glycine betaine in solid medium conferred osmotic tolerance. Though admittedly less relevant to solid foods, the effect of glycine betaine on stressed cells in liquid medium was also examined. Growth rates of cultures were determined by measuring turbidity in modified Pine's medium in the presence and absence of salt stress with and without glycine betaine. These experiments demonstrated that exogenous glycine betaine enhanced the growth of stressed cells but not of unstressed cells (Table 2). The rate was increased by more than 10-fold at 8% NaCl compared with that for unstressed cells.

Since glycine betaine accumulates intracellularly only when it is supplied in the growth medium, its accumulation must occur via transport rather than by synthesis. The question of osmotic regulation of glycine betaine transport was addressed directly by measuring the uptake of [^{14}C]glycine betaine (24). Results of uptake measurements demonstrated that the rate of glycine betaine transport was stimulated by the presence of salt in the growth medium (Fig. 1A). Maximal stimulation was

TABLE 2. Enhancement of growth of stressed *L. monocytogenes* by exogenous glycine betaine in modified Pine's liquid medium

Temperature (°C)	NaCl (%)	Growth rate (h^{-1}) (mean ± SD) at indicated GB[a] level		Fold activation[b]
		No addition of GB	130 μM GB	
30	0	0.35 ± 0.02	0.35 ± 0	1
	2	0.22 ± 0.01	0.26 ± 0.01	1.2
	4	0.13 ± 0.01	0.17 ± 0.03	1.3
	8	0.0080 ± 0.0003	0.09 ± 0.01	11
7	0	0.027 ± 0.001	0.033 ± 0.003	1.2
	2	0.020 ± 0.001	0.030 ± 0.005	1.5
	4	—[c]	0.014 ± 0.001	>3.5
4	0	0.008 ± 0	0.014 ± 0.001	1.8
	2	—[d]	0.0057 ± 0.0001	Large

[a] GB, glycine betaine.
[b] Ratio of growth rate with 130 μM glycine betaine to growth rate without added glycine betaine.
[c] Not all cultures grew under these conditions. When growth was observed, growth rates were approximately 4×10^{-3} h^{-1}.
[d] No growth in 40 days.

observed at about 4% NaCl, at which concentration the uptake rate was about 200 times faster than that observed in the absence of salt.

This increase in activity could have arisen either from activation of a constitutive enzyme or from increased synthesis of the transport system. To determine which mechanism of control is responsible for the increased uptake, cells grown in modified Pine's medium lacking betaine and without added salt were subjected to osmotic upshock in the presence of glycine betaine. Within 2 min after upshock, the cells showed a rapid increase in uptake rate (Fig. 2A) and reached a maximum rate within about 4 min. In separate experiments, cells were incubated for 2 h with either 20 or 100 μg of chloramphenicol per ml and then subjected to hyperosmotic shock. The uptake exhibited by these cells in the presence of the antibiotic was indistinguishable from that of untreated cells (data not shown), indicating that the stimulation of transport results from activation rather than from induction of the transport protein. However, the rate of uptake observed in hyperosmotic shock experiments was about 40% of that observed in cells grown under salt stress for several generations. It is thus possible that some induction does occur, although other adaptive changes that could alter the transport rate might take place in cells grown under stress.

Cold adaptation in *L. monocytogenes*. More intriguing than the osmotic tolerance exhibited by *L. monocytogenes* is its ability to grow at low temperature. Cryoprotection by the accumulation of osmotically active solutes has been reported in overwintering insects (40), in an intertidal mollusc (17), and in plants (1). Considering the ability of *L. monocytogenes* to thrive in the cold, it was of interest to determine if it also accumulates compatible solutes at low temperature. ^{13}C NMR spectra of perchloric acid extracts of cells grown in BHI broth at 30 and 4°C showed that the glycine betaine concentration was 4.7 times higher at 4 than at 30°C, while glutamate levels increased by less than twofold (Table 1).

To determine if accumulation of glycine betaine confers chill tolerance, agar plates containing modified Pine's medium with and without glycine betaine were inoculated and incubated at 7°C. After 32 days, numerous colonies (>100) were visible on plates containing glycine betaine (data not shown), whereas no colonies were visible in the absence of glycine betaine, indicating that glycine betaine stimulated growth at low temperature.

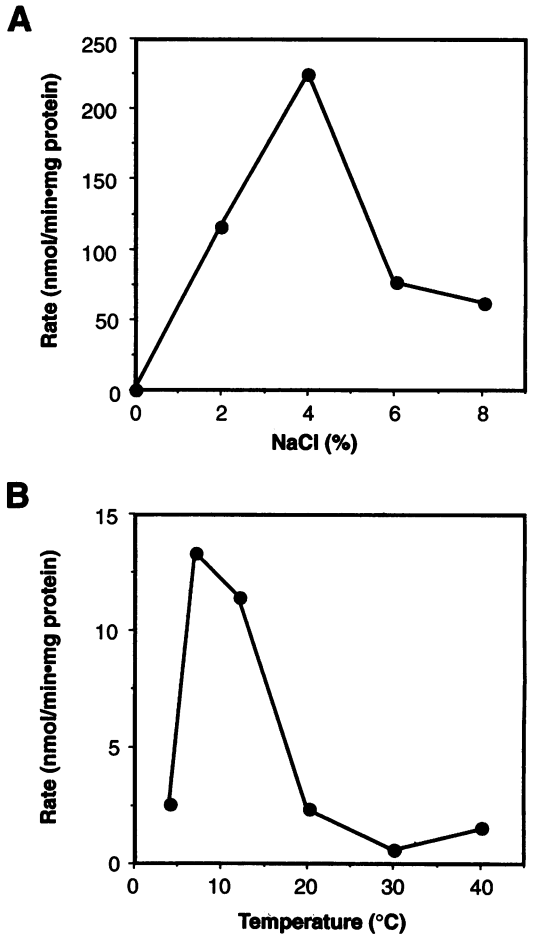

FIG. 1. Regulation of transport of glycine betaine by salt concentration and by temperature. Uptake of [^{14}C]glycine betaine (*y* axes) was measured as described in the text. (A) Cells grown at 30°C in modified Pine's medium containing the salt concentrations indicated were assayed in the same medium; (B) cells grown in modified Pine's medium at the temperatures indicated were assayed at those temperatures. Uptake rates are normalized to total cellular protein.

In modified Pine's medium, growth rate experiments at 4, 7, and 30°C with and without glycine betaine showed that glycine betaine increased the growth rate by a factor of nearly two at 4°C (Table 2).

To determine the mechanism of glycine betaine accumulation in chill-stressed *L. monocytogenes*, uptake of [^{14}C]glycine betaine was measured. The rate of glycine betaine transport was unequivocally stimulated by low temperature (Fig. 1B). Maximal stimulation occurred at about 7°C, at which temperature the rate was about 15-fold higher than that observed at 30°C.

Whether the increased glycine betaine transport was mediated by biochemical activation or by induction of the transport protein was tested using a temperature jump experiment. A shift in the temperature of cells grown at 30°C to 7°C caused rapid stimulation of transport activity within 5 min (Fig. 2B). When cells were incubated for 2 h at 30°C with 20 or 100 μg of chloramphenicol per ml and then chilled, the appearance of transport activity (not shown) was the same as that for untreated cells. Protein synthesis is therefore not required for transport. As in the case of osmotic upshock, the transport

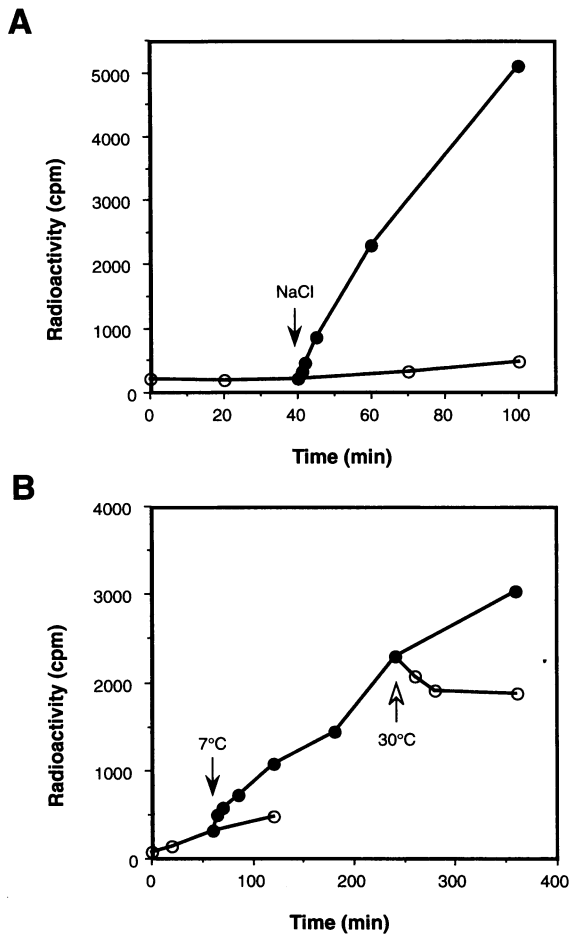

FIG. 2. Stimulation of glycine betaine transport by osmotic upshock and temperature downshock. (A) Glycine betaine transport after osmotic upshock in modified Pine's medium. The culture was divided in two, and medium containing concentrated NaCl was added to one sample to bring the concentration to 4% NaCl at the time shown by the solid arrow. Transport was measured for both stressed (●) and unstressed (○) cultures. (B) Glycine betaine transport after temperature decrease in modified Pine's medium. After incubation at 30°C (○), a portion of the culture was quickly cooled (solid arrow) to 7°C (●). At the time shown by the open arrow, a portion of the chilled sample was warmed back to 30°C (○). Raw data are shown, uncorrected for cell density. Before the temperature was decreased (0 to 60 min), the culture grew with a doubling time of 2 h, which accounts for the apparent increase in uptake during that time.

rates observed after temperature downshock were about 30% lower than in cells actually grown at low temperature, suggesting either that a modest degree of inducibility exists or that other slower adaptation processes facilitate transport of glycine betaine in cells that have been held at low temperature. When the temperature was raised to 30°C, the transport subsided and some radioactivity was lost from the cell (Fig. 2B), indicating that glycine betaine can readily be disposed of when the cell is not under chill stress. Thus, both activation and deactivation of transport are effected by temperature changes.

Kinetic parameters of glycine betaine transport. A kinetic analysis of the salt-activated and cold-activated glycine betaine transport systems yielded the Michaelis constants and maximum velocities shown in Table 3. The K_m values for salt-activated and chill-activated transport were both approximately 7 μM, suggesting that the two transport activities may be the same system. However, because the Michaelis constants are obtained under significantly different conditions (one activity must be measured at high ionic strength and the other at low temperature), they are not strictly comparable. Michaelis constants for cells grown and assayed under both stresses (7°C and 4% NaCl; Table 3) were also determined. The Michaelis constant was not decidedly different from those obtained for either stress alone. The maximum velocity was much lower than that for salt-activated transport and similar to that obtained at 7°C without added salt. This observation shows that the lower temperature can reduce the maximum velocity of salt-activated transport and that the difference in maximum velocities does not necessarily imply that the two transport activities are different.

TABLE 3. Kinetics of salt- and cold-induced glycine betaine transport[a]

Conditions[b]	K_m (μM GB)	V_{max} (nmol of GB/min · mg of cellular protein)
30°C, +4% NaCl	8.1 ± 3.4	132 ± 2[c]
7°C, no NaCl	6.6 ± 0.1	29 ± 1
7°C, +4% NaCl	2.2 ± 0.5	23 ± 1

[a] Abbreviations: GB, glycine betaine; V_{max}, maximum rate of transport. Reported errors are standard errors of at least two experiments, each run in duplicate.

[b] Reliable values could not be determined in the absence of salt and chill stress because uptake was very slow (Fig. 1) and growth rates were fast (Table 2).

[c] This value represents the uptake rate immediately after dilution of cells; the uptake rate, hence the V_{max}, increased by more than a factor of two even when corrected for cell growth during the 2 h after cell dilution. For measurement of cold-activated uptake, dilution of the cultures was not required to obtain a conveniently measured rate; the rate did not change with time.

DISCUSSION

We have observed that glycine betaine plays a critical role in osmotic and chill adaptation in *L. monocytogenes*. Our results can be compared to those obtained in other, well-studied microorganisms. *E. coli*, for example, has two glycine betaine transport systems, the products of the *proP* and *proU* loci. The ProP protein is activated by osmotic stress, as is the betaine transporter in listeria. But ProP is also weakly inducible over constitutive levels by hyperosmotic and nutritional stress. The ProU protein, however, is strongly inducible by hyperosmotic stress (8, 45) and is thought to function primarily in osmotic adaptation. In the gram-positive organism *Staphylococcus aureus*, one (2, 10) or two (28) glycine betaine transport systems have been found. When two transport systems were observed, they were distinguished by their affinities for glycine betaine, maximum velocities, and inducibility. The low-affinity system was inducible by osmotic pressure and the high-affinity system was inducible by glycine betaine itself (28), suggesting that the low-affinity system plays a role in osmotic adaptation. In contrast, the glycine betaine transport system of *L. monocytogenes*, also a gram-positive organism, is constitutive or, at most, weakly induced by NaCl in the medium.

Several of the observations reported here are particularly noteworthy. This is the first demonstration that glycine betaine confers chill tolerance in a bacterium. It is also unusual that stimulation of glycine betaine transport does not appear to involve significant induction at the genetic level in *L. monocytogenes*.

It is especially remarkable that active transport of glycine betaine is stimulated by cold. It is possible that stimulation of

TABLE 4. Inhibition of glycine betaine transport by betaine analogs and related compounds

Added compound	Inhibition (%) under indicated conditions[a]		
	30°C, 4% NaCl	7°C, no added NaCl	7°C, 4% NaCl
Dimethylglycine	55 ± 5	15 ± 2	20 ± 11
Sarcosine	43 ± 5	19 ± 4	19 ± 4
Trigonelline	57 ± 1	7 ± 5	13 ± 2
Choline	11 ± 4	0	0
Proline	9 ± 5	0	10 ± 3
Trimethylamine	0	0	0

[a] Inhibition was measured at 120 μM glycine betaine and 5 mM inhibitor. Errors represent deviations from the mean of two experiments.

glycine betaine accumulation such as that shown in Fig. 2 could occur by inhibition of efflux relative to uptake rather than by stimulation of uptake alone. However, the initial rate experiments used to determine kinetic parameters (Table 3) were initiated by the addition of labelled glycine betaine. The reaction therefore contains no significant contribution from efflux, because there is essentially no labelled glycine betaine to be exported during initial rate measurements.

The stimulation of active transport by cold is enigmatic, because low temperature is expected to impede membrane-related processes. In fact, this transmembrane transport process occurs at a temperature at which many biomembranes exist in the gel phase. The mechanism of cold activation of glycine betaine transport may involve changes in hydrophobic interactions among membrane proteins or between proteins and the membrane, because hydrophobic interactions are weakened at lower temperature.

Some organisms that are able to adapt to low temperatures maintain membrane fluidity by adjusting the composition of their membrane lipids (15, 30). Although *L. monocytogenes* may alter its membrane composition depending on the temperature at which it is grown, such adjustments would be too slow to play a role in the activation of glycine betaine transport observed in the temperature downshock experiments. However, differences in membrane composition resulting from long-term adaptation may account for the small increase in the rate of glycine betaine transport in cultures grown under cold or salt stress compared with those observed in cold-shock and osmotic-upshock experiments. Growth experiments involving either salt stress or cold stress yielded long lag phases (data not shown) which were shortened by up to 50% by glycine betaine. It is possible that other adaptation processes occur during this lag phase and that intracellular glycine betaine somehow facilitates them or protects the cellular machinery while they occur.

The exact mechanism by which glycine betaine acts as a cryoprotectant is unknown. However, betaine is considered to be a stabilizing or "salting-in" agent (46) and may function to prevent aggregation and maintain the solubility of cellular proteins or to alter the physical properties of the cell membrane (29). Osmotic stress also appears to alter membrane phase behavior (32, 44), and glycine betaine may combat both osmotic and chill stress on the membrane by a similar mechanism.

The results of inhibition experiments (Table 4) can not be used to determine whether the salt-activated and cold-activated transport systems are different. Although none of the inhibitors tested was particularly good, those effective against one system also tended to inhibit the other. Differences in the assay conditions (i.e., temperature and NaCl concentration) could alter the ratio between K_m for glycine betaine and K_i for the inhibitor enough to explain the discrepancies in the observed inhibition, assuming competitive inhibition. The relatively small degree of inhibition, even by the close analogs *N*-methylglycine (sarcosine), *N*,*N*-dimethylglycine, and choline and even at the high ratio of inhibitor to substrate, indicates high specificity of the binding site for glycine betaine. We are currently screening other possible inhibitors of this transport system as a strategy aimed at reducing the danger of food-borne listeriosis.

ACKNOWLEDGMENTS

This work was supported by USDA grant 92-37201-8092 (to G.M.S.) and NSF grants DCB-89-03923 (to L.T.S.) and DIR-90-16484 (to the University of California at Davis, NMR Facility).

We thank C. Genigeorgis and V. Razavilar for the culture of *L. monocytogenes* Scott A.

REFERENCES

1. **Aspinall, D., and L. G. Paleg.** 1981. Proline accumulation, physiological aspects, p. 205–241. *In* D. Aspinall and L. Paleg (ed.), Physiology and biochemistry of drought resistance in plants. Academic Press, Sydney, Australia.
2. **Bae, J.-H., S. H. Anderson, and K. J. Miller.** 1992. Identification of a high-affinity glycine-betaine transport system in *Staphylococcus aureus*. Appl. Environ. Microbiol. **59**:2734–2736.
3. **Brown, A., and J. Simpson.** 1972. Water relations of sugar-tolerant yeasts: the role of intracellular polyols. J. Gen. Microbiol. **72**:589–591.
4. **Coffey, T., M. Nelson, M. Bower, and B. G. Gazzard.** 1989. *Listeria monocytogenes* meningitis in an HIV-infected patient. AIDS **3**:614–615. (Letter.)
5. **Cole, M., M. Jones, and C. Holyoak.** 1990. The effect of pH, salt concentration, and temperature on the survival and growth of *Listeria monocytogenes*. J. Appl. Microbiol. **69**:63–72.
6. **Conner, D. E., R. E. Brackett, and L. R. Beuchat.** 1986. Effect of temperature, sodium chloride, and pH on growth of *Listeria monocytogenes* in cabbage juice. Appl. Environ. Microbiol. **52**:59–63.
7. **Csonka, L. N.** 1989. Physiological and genetic responses of bacteria to osmotic stress. Microbiol. Rev. **53**:121–147.
8. **Csonka, L. N., and A. D. Hanson.** 1991. Prokaryotic osmoregulation: genetics and physiology. Annu. Rev. Microbiol. **45**:569–606.
9. **Ginther, C. L., and J. L. Ingraham.** 1974. Cold-sensitive mutant of *Salmonella typhimurium* defective in nucleosidediphosphokinase. J. Bacteriol. **118**:1020–1026.
10. **Graham, J. E., and B. J. Wilkinson.** 1992. *Staphylococcus aureus* osmoregulation: roles for choline, glycine betaine, proline, and taurine. J. Bacteriol. **174**:2711–2716.
11. **Gray, M. L., and A. H. Killinger.** 1966. *Listeria monocytogenes* and listeric infections. Bacteriol. Rev. **30**:309–382.
12. **Hazel, J. R., and E. E. Williams.** 1990. The role of alterations in membrane lipid composition in enabling physiological adaptation of organisms to their physiological environment. Prog. Lipid Res. **29**:167–227.
13. **Herendeen, S. L., R. A. VanBogelen, and F. C. Neidhardt.** 1979. Levels of major proteins of *Escherichia coli* during growth at different temperatures. J. Bacteriol. **139**:185–194.
14. **James, S. M., S. L. Fanning, B. A. Agee, B. Hall, E. Parker, J. Vogt, G. Run, J. Williams, L. Lieb, T. Prendergast, S. B. Wenner, and I. Chin.** 1985. Listeriosis outbreak associated with Mexican-style cheese. Morbid. Mortal. Weekly Rep. **34**:357–359.
15. **Jones, A. L., A. C. Hann, J. L. Harwood, and D. Lloyd.** 1993. Temperature-induced membrane-lipid adaptation in *Acanthamoeba castellanii*. Biochem. J. **290**(Part 1):273–278.
16. **Kvenberg, J.** 1988. Outbreaks of listeriosis/*Listeria*-contaminated foods. Microbiol. Sci. **5**:355–358.
17. **Loomis, S. H., J. F. Carpenter, and J. H. Crowe.** 1988. Identification of strombine and taurine as cryoprotectants in the intertidal bivalve *Mytilus edulis*. Biochim. Biophys. Acta. **943**:113–118.

18. **Low, P.** 1985. Molecular basis of the biological compatibility of nature's osmolytes, p. 469–477. *In* R. Gilles and M. Gilles-Baillien (ed.), Transport processes, iono- and osmoregulation. Springer-Verlag, Berlin.
19. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. **193:**265–275.
20. **Marth, E. H.** 1988. Disease characteristics of *Listeria monocytogenes*. Food Technol. **42:**165–168.
21. **Mascola, L., L. Lieb, J. Chiu, S. Fannin, and M. Linnan.** 1988. Listeriosis: an uncommon opportunistic infection in patients with acquired immunodeficiency syndrome: a report of five cases and a review of the literature. Am. J. Med. **84:**162–164.
22. **Muller, H. E.** 1990. *Listeria* isolations from feces of patients with diarrhea and from healthy food handlers. Infection **18:**97–99.
23. **Patchett, R. A., A. F. Kelly, and R. G. Kroll.** 1992. Effect of sodium chloride on the intracellular solute pools of *Listeria monocytogenes*. Appl. Environ. Microbiol. **58:**3959–3963.
24. **Perroud, B., and D. Le Redulier.** 1985. Glycine betaine transport in *Escherichia coli*: osmotic modulation. J. Bacteriol. **161:**393–401.
25. **Pine, L., M. J. Franzus, and G. B. Malcolm.** 1986. Guanine is a growth factor for *Legionella* species. J. Clin. Microbiol. **23:**163–169.
26. **Pine, L., G. Malcom, J. Brooks, and M. Daneshvar.** 1989. Physiological studies on the growth and utilization of sugars by *Listeria* species. Can. J. Microbiol. **35:**245–254.
27. **Pinner, R. W., A. Schuchat, B. Swaminathan, P. S. Hayes, K. A. Deaver, R. E. Weaver, B. D. Plikaytis, M. Reeves, C. V. Broome, and J. D. Wenger.** 1992. Role of foods in sporadic listeriosis. II. Microbiologic and epidemiologic investigation. JAMA **267:**2046–2050.
28. **Pourkomailian, B., and I. R. Booth.** 1992. Glycine betaine transport by Staphylococcus aureus: evidence for two transport systems and for their possible roles in osmoregulation. J. Gen. Microbiol. **138:**2515–2518.
29. **Rudolph, A., J. Crowe, and L. Crowe.** 1986. Effects of three stabilizing agents—proline, betaine and trehalose on membrane phospholipids. Arch. Biochem. Biophys. **245:**134–143.
30. **Russell, N. J., and N. Fukunaga.** 1990. A comparison of thermal adaptation of membrane lipids in psychrophilic and thermophilic bacteria. FEMS Microbiol. Rev. **75:**171–182.
31. **Ryser, E., and E. Marth.** 1989. "New" food-borne pathogens of public health significance. J. Am. Diet. Assoc. **89:**948–954.
32. **Sanderson, P. W., L. J. Lis, P. J. Quinn, and W. P. Williams.** 1991. The Hofmeister effect in relation to membrane lipid phase stability. Biochim. Biophys. Acta **1067:**43–50.
33. **Schlech, W., III.** 1984. New perspectives on the gastrointestinal mode of transmission in invasive *Listeria monocytogenes* infection. Clin. Invest. Med. **7:**321–324.
34. **Schuchat, A., K. A. Deaver, J. D. Wenger, B. D. Plikaytis, L. Mascola, R. W. Pinner, A. L. Reingold, and C. V. Broome.** 1992. Role of foods in sporadic listeriosis. I. Case-control study of dietary risk factors. JAMA **267:**2041–2045.
35. **Schuchat, A., B. Swaminathan, and C. V. Broome.** 1991. Epidemiology of human listeriosis. Clin. Microbiol. Rev. **4:**169–183.
36. **Seeliger, H., and H. Finger.** 1976. *Listeria*, p. 333–349. *In* J. S. Remington and J. O. Klein (ed.), Infectious diseases of the fetus and newborn infant. W. B. Saunders Co., Philadelphia.
37. **Seeliger, H. P. R., and D. Jones.** 1984. *Listeria*, p. 1235–1245. *In* Bergey's manual of systematic microbiology, vol. 2. Williams & Wilkins, Baltimore.
38. **Smith, G. M., L. T. Smith, and R. Ko.** 1992. Salt- and cold-activated glycine betaine transport in *Listeria monocytogenes*. International Joint Meeting of the Meeting of the SEB, APS, ASZ and CSZ, Cambridge, UK, p. 57. Society for Experimental Biology, Burlington House, London.
39. **Smith, L. T., and G. M. Smith.** 1989. An osmoregulated dipeptide in stressed *Rhizobium meliloti*. J. Bacteriol. **171:**4714–4717.
40. **Storey, K. B., and J. M. Storey.** 1983. Biochemistry of freeze tolerance in terrestrial insects. Trends Biochem. Sci. **8:**242–245.
41. **Surak, J., and S. Barefoot.** 1987. Control of *Listeria* in the dairy plant. Vet. Hum. Toxicol. **29:**247–249.
42. **van der Marel, G., J. van Logtestijn, and D. Mossel.** 1988. Bacteriological quality of broiler carcasses as affected by in-plant lactic acid decontamination. Int. J. Food Microbiol. **6:**31–42.
43. **Walker, S., P. Archer, and J. Banks.** 1990. Growth of *Listeria monocytogenes* at refrigeration temperatures. J. Appl. Bacteriol. **68:**157–162.
44. **Williams, W. P., P. J. Quinn, L. I. Tsonev, and R. D. Koynova.** 1991. The effects of glycerol on the phase behaviour of hydrated distearoylphosphatidylethanolamine and its possible relation to the mode of action of cryoprotectants. Biochim. Biophys. Acta **1062:**123–132.
45. **Wood, J. M.** 1988. Proline porters effect the utilization of proline as nutrient or osmoprotectant for bacteria. J. Membr. Biol. **106:**183–202.
46. **Yancey, P., M. Clark, S. Hand, R. Bowlus, and G. Somero.** 1982. Living with water stress: evolution of osmolyte systems. Science **217:**1214–1222.